ligand that has high thermodynamic stability and one vacant coordination position for the coordinated water molecule needed for MRI. The protein-binding moiety is chosen to target the chelated metal ion to the right tissue. For imaging circulatory function, the lipophilic diphenylcyclohexyl group (Fig. 2) preferentially and reversibly binds noncovalently to human serum albumin. Gadolinium(III) complexes of multidentate aminocarboxylate ligands that are already on the market include Magnevist [Gd(DTPA)], Dotarem [Gd(DOTA)], Omniscan [Gd BMA-DTPA], and ProHance (Gd-HP-DOTA); all are extracellular imaging agents.

The naturally short half-life and appropriate particle energy of ^{99m}Tc [half-life ($t_{1/2}$) = 6 hours; maximum γ-particle energy = 2.3×10^{-14} J] have rendered it the isotope of choice for widespread clinical application in radiodiagnostic agents for many disease states (*29*). Again, the choice of ligand is driven by its kinetics, to ensure rapid complexation and uptake by the desired target tissue. One approach (*30*) is to attach a well-characterized binding molecule, such as a hormone mimic, to the radionuclide via the ligand backbone. Hence, bifunctional ^{99m}Tc-based medicinal agents are composed of four linked sections, similar to the gadolinium imaging agents: a targeting molecule, a linker, a bifunctional chelating agent, and a radionuclide. The receptor-binding motif is thus kept far apart from the technetium or indium chelate, minimizing interference between the two.

Not all lanthanide-based drugs are imaging agents; many nonetheless take advantage of particular nuclear properties of the metal ion. Commercially available ^{153}Sm-EDTMP, Quadramet, was designed to enhance tissue uptake and minimize clearance; it localizes specifically in bone. This property, together with the nuclear properties of ^{153}Sm ($t_{1/2}$ = 47 hours; maximum β-particle energy = 1.3×10^{-13} J), renders the radiopharmaceutical highly effective in alleviating the bone pain associated with metastatic bone cancer (*31*).

Prospects

The use of metal ions in medicine is not new. What is new is the increasingly purposeful design of metal-based therapeutics (*32*). Emerging possibilities for well-defined absorption, distribution, metabolism, and excretion of metal-based therapeutics will undoubtedly improve the boon/bane balance for metal ions in medicine in the coming years.

Future challenges in the field are to develop more efficient predictive methods for metal-based compounds of therapeutic interest. Varying ligand choice is one obviously verifiable way of altering the endogenous distribution of metal ions; however, no specific guidelines are available to predict the effects of variation a priori. Tissue targeting is a highly desirable goal for metal-based therapeutics or diagnostics, but it is not always feasible, and more targeting ligands must be found. For cancerous tumors, the tissue target is clear and can be biochemically differentiated from normal tissue, not least by elevated oxygen consumption. In metabolic disorders that involve multiple hormonal and enzymatic system malfunctions, such as diabetes, a more appropriate therapeutic goal may be hormonal mimicry or enhancement using metal-based drugs. These are practical issues open to solution as the field of medicinal inorganic chemistry becomes ever more interdisciplinary in nature. Empirical evidence for the utility of metal-based therapeutics has existed for centuries; theoretical understanding is bound to follow.

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REVIEW

The Ecology of Arsenic

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Arsenic is a metalloid whose name conjures up images of murder. Nonetheless, certain prokaryotes use arsenic oxyanions for energy generation, either by oxidizing arsenite or by respiring arsenate. These microbes are phylogenetically diverse and occur in a wide range of habitats. Arsenic cycling may take place in the absence of oxygen and can contribute to organic matter oxidation. In aquifers, these microbial reactions may mobilize arsenic from the solid to the aqueous phase, resulting in contaminated drinking water. Here we review what is known about arsenic-metabolizing bacteria and their potential impact on speciation and mobilization of arsenic in nature.

Despite its low crustal abundance (0.0001%), arsenic is widely distributed in nature and is commonly associated with the ores of metals like copper, lead, and gold (*1*). Arsenic can exist in four oxidation states: As(−III), As(0), As(III), and As(V). Native (elemental) arsenic occurs rarely, whereas traces of toxic arsines can be detected in gases emanating from anoxic environments (*2*). The predominant form of inorganic arsenic in aqueous, aerobic environments is arsenate [As(V) as $H_2AsO_4^-$ and $HAsO_4^{2-}$], whereas arsenite [As(III) as $H_3AsO_3^0$ and $H_2AsO_3^-$] is more prevalent in anoxic environments. Arsenate is strongly adsorbed to the surface of several common minerals, such as ferrihydrite and alumina, a property that constrains its hydrologic mobility. Arsenite adsorbs less strongly and to fewer minerals, which makes it the more mobile oxyanion (*3*). A number of methylated organoarsenicals (e.g., methylarsonic, methy-

larsonus, and dimethylarsenic acids) are found in natural waters as breakdown or excretory products from aquatic biota (*2, 4*), or as urinary excretions of animals, including humans (*5*). A recent review gives further details on the detection of various organoarsenicals in nature (*6*).

Anthropogenic point sources contribute to arsenic found in the environment. These include smelter slag, coal combustion, runoff from mine tailings, hide tanning waste, pigment production for paints and dyes, and the processing of pressure-treated wood (e.g., copper chromated arsenate). For nearly five decades (1930 to 1980), the application of arsenic-based pesticides (e.g., calcium arsenate, dimethylarsonate) alone amounted to ~10,000 metric tons per year (7). In a more isolated case, the production and storage of chemical weapons (e.g., phenyldichloroarsine, diphenylchloroarsine, diphenylcyanoarsine) has resulted in the gross contamination (>900 mg/kg) of several former mili-tary bases in Eastern Europe (8). Arsenic has been replaced in most applications by synthet-ic dyes and pesticides, but it is still used in agriculture. Organic arsenicals like roxarsone (4-hydroxy-3-nitrophenyl arsonic acid) act as an intestinal palliative for swine and prevent coccidiosis, improve pigmentation, and in-crease growth in feedlot-raised poultry (9). It has been estimated that the poultry industry on the east coast of the United States uses 20 to 50 metric tons of roxarsone annually (10). The arsenic does not accumulate in the flesh, meat, or eggs but is excreted, resulting in concentrations in excess of 20 mg/kg in ma-nure (11).

In contrast to localized sources of anthropogenic arsenic pollution, naturally occurring arsenic is very broadly distributed in many subsurface drinking water aquifers around the globe (*7*, *12*). Ironically, it is these "natural" sources that are of the most concern to human health on a global basis.

Arsenic Toxicity and Mechanisms of Microbial Resistance

The poisonous properties of arsenic compounds have been known since antiquity (*1*). Arsenic trioxide (As_2O_3) gained so much favor as a homicidal agent it was once referred to as "inheritance powder." In the mid-19th century, James Marsh devised the first chemical test for the presence of arsenic in tissue, thereby advancing forensic science while putting such nefarious heirs on notice. Indeed, the properties of arsenic have been alternatively exploited for medicinal and toxicological purposes (*1*, *13*). Arsenic trioxide is currently used as a treatment for certain forms of leukemia (*14*). The mode of toxicity depends on the chemical form of arsenic. Arsenate is a molecular analog of phosphate and inhibits oxidative phosphorylation, short-circuiting life's main energy-generation system. Its usual mode of entry is through phosphate transporters. Arsenite is even more broadly toxic because it binds to sulfhydryl groups, impairing the function of many proteins (*15*). It also affects respiration by binding to the vicinal thiols in pyruvate dehydogenase and 2-oxoglutarate dehydrogenase (*15*). More recently, it has been shown to interact with the glucocorticoid receptor (*16*). Arsenite is uncharged at pH values less than 9.2 and enters the cell via aqua-glycerolporins (*17*).

Several different mechanisms have evolved to rid cells of arsenic. These include methylation, and expulsion involving an As(III)-specific transporter. In higher eukaryotes, glutathione reduces As(V) to As(III), which then accepts a methyl group from *S*-adenosylmethionine, producing monomethylarsonic acid (MMA) or dimethylarsonic acid (DMA) (*15*). Fungi produce trimethylarsine (*18*), whereas bacteria may produce MMA and DMA (*19*). Such diverse microbes as anaerobic methanogenic Archaea (*20*) and aerobic Eubacteria (*21*) can also form methylated arsines. Arsenic may also be converted to arsenobetaine and arsenic-containing sugars, benign compounds that are found in high abundance in some marine animals and algae as well as terrestrial plants and animals (*2*, *6*).

The most well studied mechanism of detoxification and resistance, however, is the ArsC system (*17*, *22*). At least three different but structurally related arsenate reductases have convergently evolved in bacteria and yeast. ArsC, a small–molecular mass protein (13 to 16 kD), mediates the reduction of As(V) to As(III) in the cytoplasm. Although As(III) is more toxic, it can be excreted via an As(III)-specific transporter, ArsB. The *ars* operon in *Escherichia coli* has both plasmid and chromosomal loci. The plasmid R733 has four genes—*arsA*, *arsB*, *arsC*, *arsD*, and *arsR*—whereas the chromosomal locus has only *arsB*, *arsC*, and *arsR*. A cysteine residue near the N-terminal of ArsC binds the As(V), which is then reduced with electrons donated by the reduced glutathione. The As(III) is then expelled from the cytoplasm through an adenosine 5′-triphosphate (ATP)–dependent arsenite transporter formed by ArsAB (*17*). The *ars* operon in plasmid pI258 of *Staphylococcus aureus* contains only *arsB*, *arsC*, and *arsD* (*23, 24*). Reduced thioredoxin provides the electrons to reduce As(V), and As(III) is expelled from the cell via an ATP-independent ArsB. Although this process has been studied in detail in *E. coli* and *S. aureus*, it is found in many other bacteria and occurs in strict anaerobes like *Clostridium* (*25*) and *Desulfovibrio* (*26*). Arsenate reduction to As(III) has been noted in several aerobic bacteria isolated from As-contaminated soils and mine tailings (*27, 28*), suggesting that As(V) resistance plays an important role in the biogeochemical cycling of this element in nature (*29*).

Dissimilatory Arsenate-Reducing Prokaryotes

Considering the toxicity of arsenic to both prokaryotes and eukaryotes, the discovery that As(V) serves as a "nutrient" to certain anaerobes by functioning as their respiratory oxidant came as a surprise. The reaction is energetically favorable when coupled with the oxidation of organic matter because the As(V)/As(III) oxidation/reduction potential is +135 mV. Two closely related representatives of the ε-Proteobacteria, *Sulfurospirillum arsenophilum* and *Sulfurospirillum barnesii*, were the first microbes reported that could achieve this feat (*30–32*). Both conserve energy by linking the oxidation of lactate to the reduction of As(V) to As(III) [Gibbs free energy ($\Delta G°$) = −295 kJ/mol lactate]. At present there are at least 16 species in pure culture, and include representatives from the γ-, δ-, and ε-Proteobacteria, low-GC Gram-positive bacteria, thermophilic Eubacteria, and Crenoarchaea (Fig. 1). We collectively refer to these microbes as dissimilatory arsenate-reducing prokaryotes (DARPs). They have been isolated from freshwater sediments, estuaries, soda lakes, hot springs, and gold mines [reviewed in (*33*)]; the gastrointestinal tracts of animals (*34*); and subsurface aquifer materials from Bangladesh (*35*). They include several extremophiles adapted to high temperature, pH, and/or salinity (*36–38*). These organisms can use a variety of electron donors including hydrogen, acetate, formate, pyruvate, butyrate, citrate, succinate, fumarate, malate, and glucose (*39*). Recently, some strains have been found to degrade more complex aromatic molecules like benzoate and even toluene (*23*). Certain species are more sensitive to arsenic than others. Whereas the haloalkaliphile *Bacillus selenitireducens* grows well at 10 mM As(V), possibly because the product As(III) is charged at high pH and cannot enter the cell, *Sulfurospirillum* species grow best at 5 mM. To date, no "obligate" DARPs have been found, because all the strains examined can use other electron acceptors for growth. For example, *Desulfotomaculum auripigmentum* (*24*) and *Desulfomicrobium* strain Ben-RB (*26*) also respire sulfate. *S. barnesii* is the most versatile, because it also respires selenate, nitrate, nitrite, fumarate, Fe(III), thiosulfate, elemental sulfur, dimethylsulfoxide, and trimethylamine oxide (*31, 40*). This metabolic diversity may be an important ecological factor, because sulfur, iron, and nitrate

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chemical species [e.g., S(−II), FeOOH, NO_3^-] interact with arsenic in the environment.

Although the biochemistry of respiratory As(V) reductases remains to be fully elucidated, it is clear that they differ both functionally and structurally from ArsC. The respiratory arsenate reductase from *Chrysiogenes arsenatis* is a heterodimer consisting of subunits of 87 and 29 kD and is located in the periplasm (*41*). N-terminal sequence data suggest that both subunits contain an iron-sulfur cluster, placing this protein in the dimethylsulfoxide (DMSO) reductase family of mononuclear molybdenum enzymes. Initial investigations of the As(V) reductase from the Gram-positive bacterium *B. selenitireducens* revealed similar characteristics. N-terminal sequence analyses indicate a 50% sequence identity and 85% similarity of both ArrA and ArrB subunits of *C. arsenatis* (*42*). The putative arsenate reductase from *S. barnesii* is also believed to be oriented in the periplasm, but it consists of a single subunit (48 kD) and has no metal associated with it (*43*). Enzymological and immunological analyses further indicate notable differences in the enzyme from *S. barnesii* and related *Sulfurospirillum* species (*S. arsenophilum*, *S. deleyianum*). The ability to respire arsenate does not preclude the presence of a separate, arsenate-resistance system as well. Recently, *Shewanella* strain ANA-3 was found to have both respiratory and detoxifying arsenate reductases (*44*).

The environmental impact of DARPs has only recently been realized (*45*–*50*). Their activity can be readily discerned using incubations of anoxic sediment slurries amended with millimolar (1 to 5) arsenate (*46*). Most-probable-number determinations of sediments from arsenate-contaminated lakes indicate resident populations of between 10^4 and 10^5 cells per gram (*48*, *51*). The process of dissimilatory As(V) reduction occurring in near-surface hyporheic zones greatly affects the transport and speciation of arsenic in freshwater streams (*52*). DARPs can also attack As(V) adsorbed to solid phases like ferrihydrite and alumina (*45*) and reduce the As(V) contained in oxidized minerals like scorodite (*24*, *47*). This latter point contrasts with findings from studies done with nonrespiratory arsenic-reducing bacteria that showed release of adsorbed As(V) as a result of iron reduction (*53*) or negligible release of As(V) and no dissolution of the mineral substrate (*54*).

Although considered negligible in most environments, the role of DARPs in the oxidation of autochthonous organic matter can be appreciable in specific cases. In situ measurements of arsenate respiration in Mono Lake, California (a particularly arsenic-rich environment; dissolved inorganic arsenic = 200 μM), made with the radiotracer $^{73}As(V)$, revealed that as much as 14% of annual primary productivity was mineralized to CO_2 in the anoxic water column by the activity of DARPs (*49*). In the anoxic water column of Mono Lake, DARPs number between 10^2 and 10^3/ml. These numbers appear to be low, probably because the method requires that they achieve growth in the medium provided. Culture-independent polymerase chain reaction (PCR) techniques to enumerate DARPs have not yet emerged, in part because their diverse phylogeny negates the utility of commonly used 16*S* ribosomal DNA probes and because DARPs isolated thus far are opportunists capable of respiring electron acceptors other than arsenate. Denatured gradient gel electrophoresis (DGGE) of DNA extracted from anoxic Mono Lake water incubated with 1 mM As(V) resolved bands suggesting that members of the ε- (*Thiomicrospira*) and δ-Proteobacteria (*Desulfovibrio*) might be contributing to arsenate respiration in these waters (*50*). In contrast, DGGE resolution of in situ DNA from bottom water indicated that the *Bacillus* and *Clostridia* genera were the dominant population (*55*). Because the arsenate-respiring *Bacillus arsenicoselenatis* and *B. selenitireducens* species were originally isolated from Mono Lake's bottom sediments (*36*), they may typify most of the DARPs present in the water column.

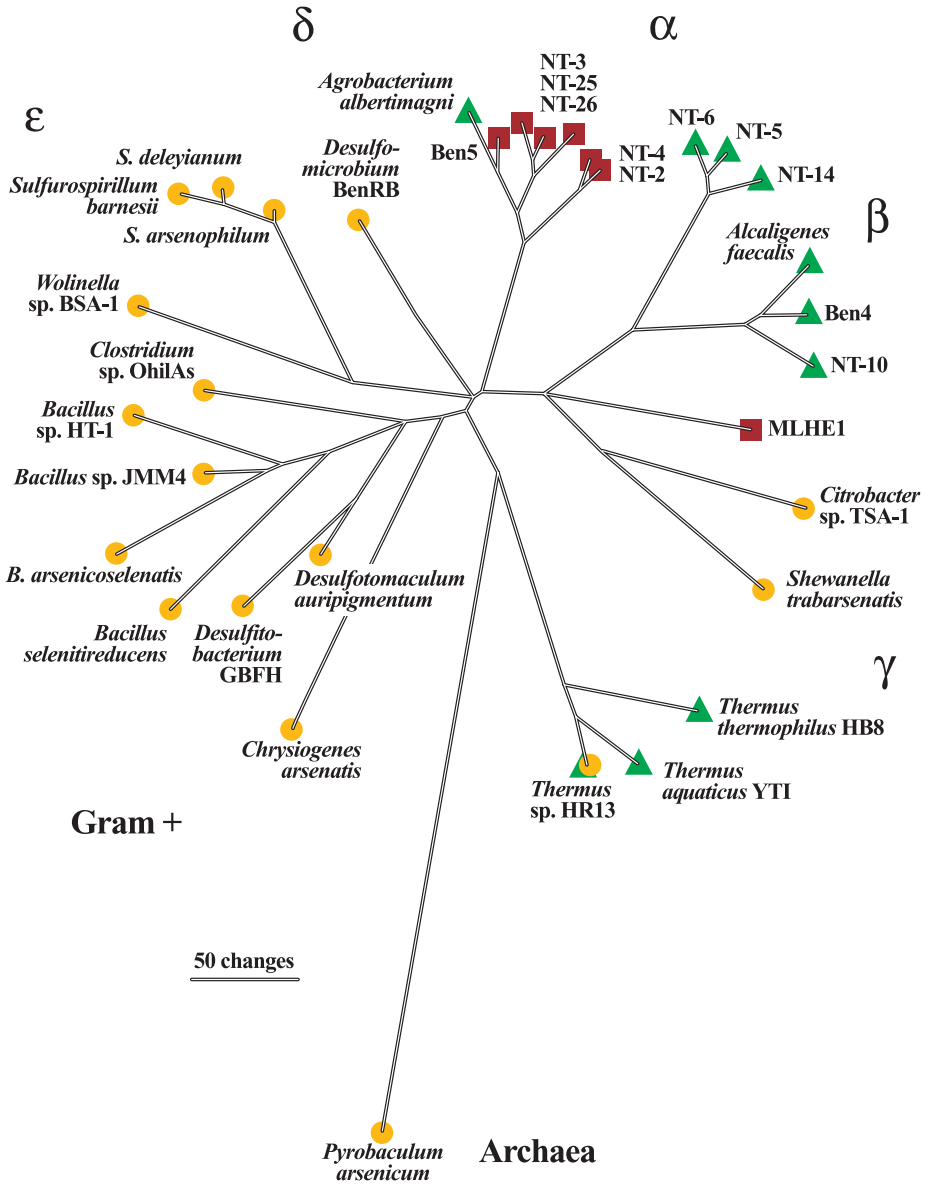

Fig. 1. Phylogenetic diversity of representative arsenic-metabolizing prokaryotics. Dissimilatory arsenate-respiring prokaryotes (DARPs) are indicated by yellow circles, heterotrophic arsenite oxidizers (HOAs) are indicated by green triangles, and chemoautotrophic arsenite oxidizers (CAOs) are indicated by red squares. In some cases (e.g., *Thermus* sp. strain HR13), the microbe has been found able to both respire As(V) and oxidize As(III).

Arsenite-Oxidizing Prokaryotes

The microbiological oxidation of As(III) to As(V) can also impact the mobility and speciation of arsenic in the environment. The process has been known for many years (56), and more than 30 strains representing at least nine genera have been reported to be involved, including α-, β-, and γ-Proteobacteria; Deinocci (i.e., Thermus); and Crenarchaeota (Fig. 1). Physiologically diverse, they include both heterotrophic arsenite oxidizers (HAOs) and the more recently described chemolithoautotrophic arsenite oxidizers (CAOs). Heterotrophic oxidation of As(III) is viewed primarily as a detoxification reaction that converts As(III) encountered on the cell's outer membrane into the less toxic form, As(V), perhaps making it less likely to enter the cell. CAOs couple the oxidation of arsenite (e.g., electron donor) to the reduction

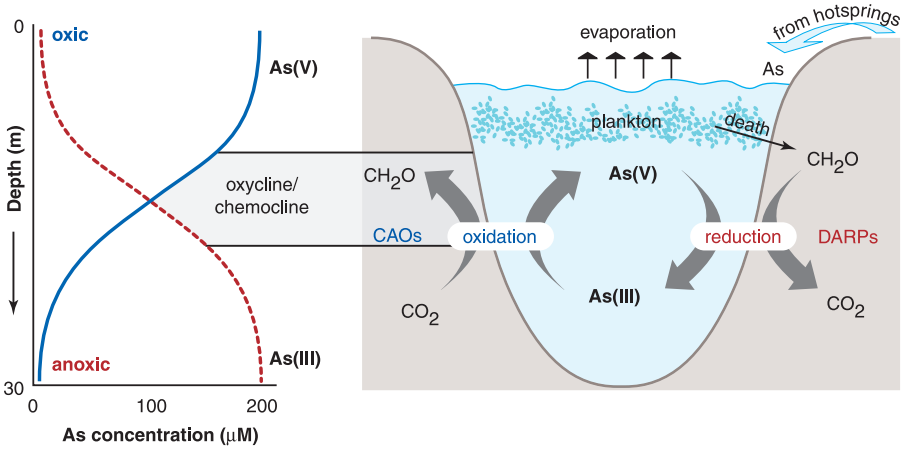

Fig. 2. The chemical speciation of arsenic in the stratified water column of Mono Lake, California (**left**) as explained by the metabolism of arsenic by microbial populations present in the water column (**right**). Arsenic cycling occurs in the region of the chemocline. Arsenate reduction is mediated by DARPs that use released organic matter from dying plankton to fuel their respiration. Arsenite oxidation (aerobic and anaerobic) is mediated by CAOs that also contribute to secondary production by "fixing" CO_2 into organic matter. Arsenic first enters this alkaline (pH = 9.8), saline (90 g/liter) lake as a dissolved component contained in the discharge from hydrothermal springs. Arsenic, as well as other dissolved constituents, reaches high concentrations because of the predominance of evaporation over precipitation in this arid region.

of either oxygen or nitrate and use the energy derived to fix CO2 into organic cellular material and achieve growth. In HAOs the oxidation of As(III) is catalyzed by a periplasmic enzyme that is distinct from the dissimilatory arsenate reductase. This mononuclear molybdenum enzyme, belonging to the DMSO reductase family, is structurally similar to the periplas-mic nitrate reductase (NapA) from Desulfo-vibrio desulfuricans (57). It is a heterodimer, with a catalytic subunit (85 kD) that con-tains molybdenum bound to two pterin cofac-tors and a [3Fe-4S] cluster. The associated subunit (14 kD) presumably functions as an electron shuttle and has a Rieske-type [2Fe-2S] cluster, a feature that is unique among molybdenum enzymes (58). The arsenite oxi-dases of CAOs, however, remain to be fully characterized

Arsenite oxidation is being studied as the basis for bioremediation of systems where As(III) is a pollutant, because the As(V) can be immobilized onto strong adsorbents (59). Interest in this subject has resulted in the recent isolation of several novel species of both heterotrophic and autotrophic aerobic As(III) oxidizers from arsenic-rich environ-ments (60, 61). Strain NT-26, a fast-growing CAO, is a member of the Rhizobium clade of the α-Proteobacteria and grows either by che-moautotrophic As(III) oxidation or as a con-ventional heterotroph by using organic com-pounds in lieu of As(III) (62). Gihring and Banfield (38) isolated a curious thermophilic species of Thermus (strain HR 13) from an As-rich hot spring. Under aerobic conditions it will oxidize As(III) for detoxification pur-poses without conserving the energy pro-duced by the reaction. However, under anaer-obic conditions, strain HR 13 can grow on lactate using As(V) as its electron acceptor. Field studies have demonstrated that microbial oxidation of As(III) occurs along reaches of arsenic-rich geothermal streams (*63*), and mo-lecular techniques have been used to identify arsenite-oxidizing populations (HAOs) of ther-mophilic prokaryotes present in various hot springs of Yellowstone National Park (*64*).

Recently, a novel species of the *Ectothio-rhodospira* clade of Eubacteria was isolated from Mono Lake that grew under anaerobic conditions using As(III) as its electron donor and nitrate as its electron acceptor:

$$H_2AsO_3^- + NO_3^- \rightarrow HAsO_4^{2-} + NO_2^- + H^+$$

$$\Delta G^\circ = -56.5 \text{ kJ/mol}$$

This nonphotosynthetic bacterium, strain MLHE-1, also grew as an autotroph with sulfide or hydrogen gas in lieu of As(III), and additionally grew as a heterotroph on acetate with oxygen or nitrate as the electron acceptor (*65*). Curiously, it was unable to grow on or oxidize As(III) under aerobic conditions. The occurrence of anaerobic arsenite oxidation suggested that there might be a tight coupling between respiratory reduction of As(V) at the expense of electron donors like organic compounds and H_2, and its resupply as carried out by microbial As(III) oxidation at the expense of commonly occurring strong oxidants like nitrate, nitrite, or perhaps Fe(III). Such a theoretical coupling is illustrated in Fig. 2 for a stratified system like Mono Lake, in which the abundance of arsenic in the lake is from natural hydrothermal inputs coupled with evaporative concentration. Mono Lake is an "extreme" environment in terms of its high pH (9.8), high salinity (90 g/liter), and high content of other toxic minerals. Recently, nitrate-linked microbial oxidation of arsenite was shown to occur in an arsenic-contaminated freshwater lake (*66*), and injection of nitrate into a subsurface aquifer resulted in the immobilization of arsenic (*67*). Thus, this phenomenon appears to be widespread in nature. It remains to be determined what types of microorganisms carry out this reaction in freshwater or marine systems, as compared with those found in soda lakes.

Environmental Impacts of Microbial Arsenic Transformations

The contribution made by microorganisms to the biogeochemistry of arsenic in the environment is extensive and detailed as it involves various oxidation, reduction, methylation, and demethylation reactions of its dominant chemical species. Unlike sulfur, where volatile organic species can play a crucial role in its biogeochemical cycle, it is apparent that natural organoarsenicals do not contribute substantially in this regard. However, from an ecological perspective, we can limit this scope to consider only the flow of energy linked to arsenic metabolism that translates into a capacity to do biological work (i.e., cell growth). We therefore consider the "ecology" of arsenic to be simple in the sense that it is predominantly confined to microbial transformations between its +3 and +5 oxidation states, constrained further by considering only those prokaryotes that conserve the energy associated with these redox reactions to achieve growth. Although energy-yielding biochemical reactions mediating the oxidation or reduction of the 0 or –3 oxidation states of arsenic may be possible, they have not been observed. Regardless of the simplicity of the cycle, understanding the role of microorganisms in the hydrologic mo-

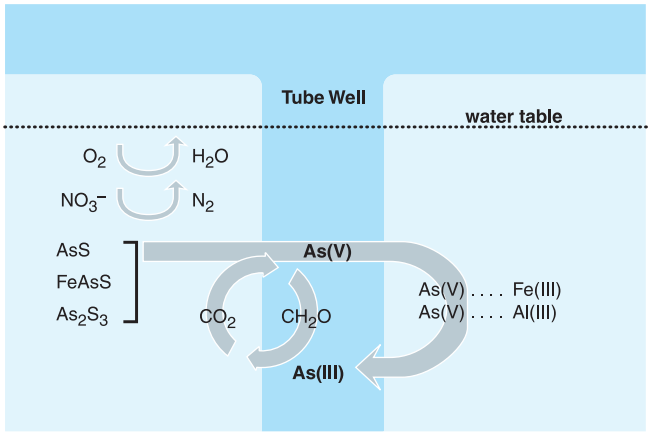

Fig. 3. A conceptual model of how arsenic-metabolizing prokaryotes may contribute to the mobilization of arsenic from the solid phase into the aqueous phase in a subsurface drinking water aquifer. Arsenic is originally present primarily in the form of chemically reduced minerals, like realgar (AsS), orpiment (As_2S_3), and arsenopyrite (FeAsS). These minerals are attacked by CAOs, which results in the oxidation of As(III), as well as iron and sulfide, with the concurrent fixation of CO_2 into organic matter. Construction of wells by human activity accelerates this process by providing the necessary oxidants like molecular oxygen or, in the case of agricultural regions, nitrate. The As(V) can subsequently be adsorbed onto oxidized mineral surfaces like ferrihydrite or alumina. The influx of substrate organic materials derived either from buried peat deposits, recharge of surface waters, or the microbial mats themselves promotes microbial respiration and the onset of anoxia. DARPs then respire adsorbed As(V), resulting in the release of As(III) into the aqueous phase.

bility of arsenic in drinking water aquifers is a highly complex but unresolved environmental question that is of critical importance to the health of millions of people worldwide. Factored into such complexity are the competing chemical reactions that affect both the speciation and the partitioning of arsenic between the aqueous phase and the solid mineral phase of the aquifer matrix (*68*). In Bangladesh alone, perhaps 30 million people drink well waters that contain elevated arsenic concentrations, and thousands of new cases of severe arseniasis (arsenicosis) occur annually in that country (*69*, *70*).

Several theories have been proposed to explain the subsurface mobilization of arsenic. These include (i) the oxidation of As-containing pyrites (*71*), (ii) the release of As(V) from reduction of iron oxides by autochthonous organic matter (e.g., peat) (*72*), (iii) the reduction of iron oxides by allochthonous organic matter (from dissolved organics in recharging waters) (*67*), and (iv) the exchange of adsorbed As(V) with fertilizer phosphates (*73*). In light of our above discussion of microbes that metabolize arsenic, we suggest that these are not necessarily mutually exclusive processes, but that over time microorganisms probably play an essential role in both the direct reduction and oxidation of the arsenic species, as well as the iron minerals contained in these aquifers. On the basis of what we now know is possible with regard to the microbial metabolism of arsenic in nature, we can begin to formulate a conceptual model for what might be occurring in the aquifers of Bangladesh. Perhaps the initial process is the oxidation of the original As(III)-containing minerals (e.g., arsenopyrite) during transport and sedimentation by pioneering CAOs and HAOs taking place over recent geologic time periods. This would result in the accumulation of As(V) onto surfaces of oxidized minerals like ferrihydrite. Subsequent human activity in the form of intensive irrigated agriculture, digging of wells, and lowering of groundwater tables would provide oxidants (e.g., oxygen, nitrate) that would further stimulate As(III) oxidation. This would cause a buildup of microbial biomass (and its associated organic matter) and the creation of anoxic conditions. This organic matter, in conjunction with other sources either from decomposing buried peat deposits or from that dissolved in seasonal recharge from agricultural surface waters, would in turn promote the dissimilatory reduction of adsorbed As(V) by DARPs and the eventual dissolution of adsorbent minerals like ferrihydrite. The end result of these processes acting in concert over time and accelerated by human activities would be the release of arsenic into the aqueous phase, as illustrated in Fig. 3. Indeed, preliminary evidence suggests the presence of an anaerobic, microbial arsenic cycle in the subsurface aquifers of Bangladesh. Injection of nitrate into the aquifer promoted the rapid removal of As(III) (*67*), which indicates the presence of a community of microorganisms similar in physiology to MLHE-1. In addition, DARPs have been cultured from As-contaminated Bangladesh aquifer sediments (*35*).

Future Research Directions

Although there is an immediate research need for a fuller understanding of the role(s) of subsurface microbes in mobilizing arsenic in aquifers, on a more speculative level, it is tempting to contemplate a microbial "biome" supported by arsenic cycling. Indeed, it can be argued that because arsenic is a "chalcophilic" (sulfur-loving) element, it should be more abundant in the Earth's interior than in its crust, and possibly more abundant on the surface of less differentiated, volcanically active planetary bodies like Mars and Europa (*74*). Provided that liquid water was present, and that there were also oxidants available that were stronger than As(V) to recycle As(III) (e.g., nitrate), Mars or Europa could conceivably have evolved primitive microbial ecosystems based in part upon use of arsenic as an energy source (*64*). Although such speculation on our part certainly borders on the fanciful, it also poses the more relevant question, how did prokaryotes on Earth evolve enzyme systems that are capable of exploiting the energy to be gained by reducing or oxidizing inorganic arsenic? Are these ancient systems dating back to the anoxic Archaean era of some 3.5 billion years past, when noxious substances were abundant on this planet's surface and the ability to exploit them for energy gain may have conferred some selective advantage? Conversely, are they more recent in origin and reflective of the need for an oxidizing atmosphere and strong oxidants to recycle As(III)? Does the wide phylogenetic distribution of DARPs among the prokaryotes (Fig. 1) indicate a long vertical evolution from one original gene, a convergent evolution of several independent genes, or merely a high degree of lateral gene transfer of a useful trait? Future research on the biochemistry of dissimilatory arsenate reductases and their analogous arsenite oxidases, and the genes that encode the proteins of the diverse (and growing) list of microorganisms, may ultimately reveal the answers.

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REVIEW

The Biogeochemical Cycles of Trace Metals in the Oceans

F. M. M. Morel[1]* and N. M. Price[2]

Planktonic uptake of some essential metals results in extraordinarily low concentrations in surface seawater. To sequester or take up these micronutrients, various microorganisms apparently release strong complexing agents and catalyze redox reactions that modify the bioavailability of trace metals and promote their rapid cycling in the upper water column. In turn, the low availability of some metals controls the rate of photosynthesis in parts of the oceans and the transformation and uptake of major nutrients such as nitrogen. The extremely low concentrations of several essential metals are both the cause and the result of ultraefficient uptake systems in the plankton and of widespread replacement of metals by one another for various biochemical functions.

The phytoplankton of the oceans are responsible for about half the photosynthetic fixation of carbon (primary production) on Earth (*1*). In contrast to most land plants, which grow relatively slowly and contribute only a small percentage of their biomass to the terrestrial food chain on any given day, marine phytoplankton divide every day or every week to keep up with zooplankton grazers. To do this, they must take up from seawater—along with carbon, nitrogen, phosphorus, and silicon (for diatoms)—a suite of essential micronutrients that are present at trace concentrations (<0.1 μM). To make matters worse, these organisms impoverish their own milieu because the elements they require for growth are continuously exported out of the sunlit surface as settling organic biomass. In comparison, terrestrial plants, which can acquire nutrients from soil and recycled litter, have a bountiful life. With regard to essential micronutrients, the ocean, particularly far from land, is the most extreme environment for life on Earth.

How does this system work? How do planktonic organisms acquire micronutrients and control their availability? To what extent does the low availability of these nutrients control the rate of enzymatic reactions, the productivity of the oceans, and the biogeochemical cycles of elements such as carbon and nitrogen? These are questions that oceanographers can now pose as testable hypotheses and can begin to answer.

Low Surface Concentrations of Essential Metals

A dozen or so elements with atomic mass above 50 are known to have a biological role, often as cofactors or part of cofactors in enzymes and as structural elements in proteins. Of those, the trace metals—Mn, Fe, Co, Ni, Cu, Zn, and Cd—have been best studied by oceanographers (*2*) and are the focus of our discussion. They are present in the plankton biomass at concentrations ranging from about 50 μmol/mol C (1000 μM) for Fe, which is used in a number

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